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Cadmium (Cd)-concentration and histopathological changes and metallothionien expression in the kidney of wild great bandicoots residing in a Cd-intoxicated area in Thailand

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Abstract

The cadimium (Cd)-concentration and the gene expression of metallothionien, an intrinsic Cd-detoxifying protein, as well as the histopathology were examined in kidneys of wild great bandicoots from Cd-contaminated and non-contaminated areas in Thailand. The Cd-concentration was higher in the animals with higher body-weight/size from both of the contaminated and non-contaminated areas. The Cd-concentration in large-sized animals from the contaminated area was the highest among all the specimens, while that in medium-sized animals from the contaminated area was higher than that in large-sized ones from the non-contaminated area. No significant difference in the Cd-concentration was noted between small-sized animals from the contaminated and non-contaminated areas. In accord with the comparison result of the renal Cd-concentration, the histopathological changes such as necrosis, inflammatory cells infiltration and fibrosis, were more severe in the kidneys of animals with larger body-sizes from the contaminated areas. No significant difference in the expression of gene for metallothionien was noted between kidneys of the animals from the contaminated and non-contaminated areas.

Keywords: cadmium, wild great bandicoot, kidney, light microscopy, metallothionien expression, weight-size subgrouping

1. Introduction

Cadmium (Cd) is one of influential environmental toxic heavy metals which exert deteriorating effects on the human health. The degree of Cd levels in the environment depends largely on the presence of Cd-implicated industrial facilities which could result in a significant increase of its concentration in the environmental air, water and soils, and further result in an involvement of Cd in the food-chain. Therefore, human exposure to Cd mainly occurs by inhalation and ingestion [1]. The rate of Cd-absorption after inhalation depends on the size and solubility of its particles and it varies up to 50% of the amount inhaled. The average rate of Cd-absorption after ingestion is approximately 5%, but it increases considerably in cases of calcium- or iron-deficiency and may reach a value up to at least 20% [2]. The exposure to Cd thus causes various deteriorations in many organs, such as irritation of the respiratory system, skeletal tissue damages, hepatotoxicity and nephrotoxicity [3]. It is known that Cd selectively accumulates in the proximal tubular epithelial cells [4], and the level of Cd-accumulation in the renal tubular cells intimately relates to the nephrotoxicity, resulting in the renal dysfunction [5].

Metallothionien (MT) is a low molecular weight protein with a high content of cysteine [6]. The expression of gene for MT occurs intrinsically in many tissues of animal body, and it is enhanced readily by various factors, especially by metal ions including Cd [7]. MT plays roles not only in normal absorption, transport, and excretion of heavy metals, but also in protection of the organisms from intoxication of toxic heavy metals such as Cd (8). In the kidney, the accumulation of Cd activates MT synthesis in the renal epithelial cells and MT binds with Cd ions to form Cd-MT complex, resulting in detoxification of Cd [6]. Therefore, MT is regarded as a Cd-inducible and thus intrinsic protein curable for Cd-intoxication. The increase in urinary MT protein was found in populations exposed to Cd, which leads to consider the possibility that MT is an indicator of both Cd-exposure and kidney dysfunction [9].

Mae Sot District represents the first reported area of environmental Cd pollution in Thailand. The paddy field receives the irrigation from two creeks, Mae Tao Mai and Mae Ku, both of which pass through a zinc-rich area where zinc mines have been actively operated for more than 20 years [10]. The concentration of Cd in soils of this area was reported to range from 0.5 to 284 mg/kg [11], which is up to 1,800 times higher than the officially permissible level of Cd in soils, 0.01-0.17 mg/kg (12). The Cd-concentration of rice grains was also reported to range between 0.05 and 7.7 mg/kg in the area [11], with over 95% of the samples having the Cd-content above the level considered officially to be "safe" for staple foods (0.1 mg/kg) [13]. Therefore, all the organisms including plants, animals and humans in the Cd-contaminated area could intake Cd by inhalation of Cd-polluted air and consume Cd accumulated in the food-chains. Thus, it is possible that the risk of chronic Cd-accumulation and toxicity is present in humans and wild animals which have been naturally exposed to Cd in Mae Sot district.

In order to understand the accurate state of Cd-intoxication in humans under actually changeable conditions in the natural ecosystem, it is useful and dispensable to examine by the field-study the accumulation of Cd in wild animals residing in areas environmentally exposed to Cd, in addition to the examination of experimentally Cd-intoxicated animals. The present study addressed this issue and selected wild great bandicoots (*Bandicota indica*) as the target to be examined because of their common occurrence in high population densities, their habit of residing in rather restricted areas, and their relatively short life and easiness to be caught. The species-determination was done using the taxonomic identification key [14].

The present study clarified various degrees of histopathological changes and the gene expression levels of metallothionein in the kidney of the wild animals from the Cd-contaminated area, Mae Sot district, Tak, Thailand, and from a Cd-non-contaminated control one, Huai Mek district, Kalasin, Thailand. The significance of differences in Cd-concentration in the animals between the two areas and the possibility of wild great bandicoot kidney as the bioindicator for the human Cd-intoxication were discussed.

2. Materials and methods

2.1. Animals

Twenty one male local wild great bandicoots (*Bandicota indica*) from Huai Mek district, Kalasin were randomly trapped during September and October 2010, and classified as the Cd- non-contaminated control animal group, whereas seventy male wild great bandicoots from Mae Sot district, Tak, were trapped during the same period and classified as the Cd-contaminated group. The animals in each group were divided into 3 subgroups based on the body weight, eye color, and skin color with characteristic of hair color and thickness to clarify as the different age groups: small (S)-subgroup including animals weighing 150~250g, medium (M)-subgroup including animals weighing 300~400g; and large (L) subgroup including animals weighing 450~600g. This grouping was made with the assumption that the body weight-size represents the ontogenic differences, although the estimation of the ages of animals on the basis of eye lens-weight was reported by Boonsong *et al*., (1988) [14]. The numbers of Cd-non-contaminated animals in S-, M- and L-size were 6, 8, and 10, respectively. While the numbers of Cd-contaminated animals in the S-, M- and L-size were 37, 16, and 17 respectively. No statistically significant differences were found in the average body weight of animals in corresponding subgroups between Cd-non-contaminated and Cd-contaminated groups ($P > 0.05$).

2.2. Soils and Rice grains

Soil samples and rice grains were collected at the center and at several radiate random sites about 1 km away from the center of the contaminated area, and at the center of the non-contaminated area. Animals were trapped within circle areas with 2 km-diameter whose centers were the area- centers. All samples were then measured the Cd concentrations by followed the previous protocols [11] & [12]. The contents in soils and rice grains of some other metals inducible of intoxicated contamination such as arsenic, mercury, lead, and copper were also examined to exclude the possibility that the obtained pathological changes were induced by them.

2.3. Specimen preparation

Animals, under anesthesia by intraperitoneal injection of 60 mg/kg body weight of sodium pentobarbital, were transcardially perfused with physiological saline for 5 min. Right kidneys were extirpated and they as well as soils and rice grains were processed for measurement of the Cd-concentration by Atomic Absorption Spectrometry (AAS; Perkin-Elmer 1100) in its recommended protocol. While upper halves of left kidneys were immersed in

4% paraformaldehyde in 0.1 M PBS (pH 7.2) for 6 hr and processed for paraffin-embedding and hematoxylin & eosin (H&E) staining, their lower half was used for reverse transcriptase polymerase chain reaction (RT-PCR) analysis to examine the gene expression of metallothionien (MT). In light microscopy of H&E-stained preparations, at least 5 sections per each kidney specimen were observed and photographed under an Olympus BX50 microscope (Olympus; Tokyo, Japan).

2.4. Metallothionien gene expression

Random samples of cell lysates from the renal tissues were used to analyze MT1A and MT2A genes expression by RT-PCR method. RT-PCR was carried out following the protocol reported by Sakagami *et al*., (2006) [15]. The primer sets used for RT-PCR were as follows. Nucleotide primers for MT1A: 5´ AGA CGC GCA AAT AAA TGT CC 3´ for forward direction and 5´ AGA ACT CCA GGG AGC CTA GC 3´ for reverse direction with the product size of 190 bp; MT2A primers: 5´ ACA GAT GGA TCC TGC TCC TG 3´ for forward direction and 5´ AAG TGT GGA GAA CCG GTC AG 3´for reverse direction with the product size of 248 bp. Rat β-actin primers: 5'-GGC TGT GTT GTC CCT GTA TG-3' for forward direction and 5'-ACT GTG TTG GCA TAG AGG TC-3' for reverse direction with the product size 483 bp were used as a house-keeping gene control. The cDNA template was amplified with the specific primers. The PCR products were electrophoresed, and then the expression of MT1A and MT2A genes were detected. The relative densities of MT1A and MT2A gene expressions were measured by Scion image software. The amount of gene expression for a given molecule was represented by relative intensity to corresponding β-actin expression.

2.5. Statistical analysis

All data are expressed as means ± standard errors of mean (mean ± SEM) values. Independent t-test was used for statistical comparison between the Cd-contaminated and Cd-non-contaminated groups. Significance was accepted with $P < 0.05$.

3. Results and discussion

3.1. Cd concentrations in rice grains and soils

The Cd-concentrations of rice grains from the Cd-contaminated area were 2.065 mg/kg and 0.146 mg/kg, whereas the concentration in the Cd-non-contaminated area was 0.026 mg/kg. On the other hand, the Cd-concentrations of soils from the Cd-contaminated area were 0.217 mg/kg, 0.245 mg/kg, 3.117 mg/kg, 16.768 mg/kg, 28.531 mg/kg, 28.056 mg/kg, 38.898 mg/kg and 49.008 mg/kg. In contrast, the Cd-concentrations of soils from the Cd-non-contaminated area were 0.004 mg/kg, 0.008 mg/kg, 0.01 mg/kg, 0.011 mg/kg, 0.019 mg/kg and less to non-detectable levels. The concentration of the other intoxicable metals such as arsenic, mercury, lead and copper were not exceeded their control levels (data not shown).

3.2. Cd concentration in kidney

The concentration of Cd in the kidney of each subgroup was shown in Table 1. While the renal Cd-concentration tended to increase with the body weight in both Cd- non-contaminated and Cd-contaminated groups, the increase was remarkable in the Cd-contaminated group with the highest concentration of the Cd-contaminated large-sized subgroup among the total six subgroups (1,800.77 ± 421.94 µg/kg, with statistical significance at $p = 0.002$). The concentration of the Cd-contaminated medium-sized subgroup was much higher than the Cd-non-contaminated counterpart (972.00 ± 250.92 µg/kg versus 102.87 ± 38.19 µg/kg, respectively, with statistical significance at $p = 0.002$), and it was even higher than that of the Cd-non-contaminated large-sized subgroup (696.78 ± 148.96 µg/kg, with statistical significance at $p = 0.002$). The concentration of the small-sized subgroups was quite low in both Cd-contaminated and Cd- non-contaminated groups without significant differences (70.81 ± 21.35 µg/kg and 73.54 ± 8.45 µg/kg, respectively, $p > 0.05$).

3.3. Histopathology of kidney

In general accord with the findings already reported by others in experimentally Cd-intoxicated animals [16], [17], [18] & [19], major pathological changes in the present study were the necrosis, the inflammation and the fibrosis of the renal tubule (Figure. 1). The severity of the individual pathological changes was divided into four grades ranging from 0 to 3 based on following criteria: Grade 0 represented actual absence of any pathological changes, grade 1 represents a situation that less than 25% of observed areas were lesioned by given pathological changes, grade 2 represents a situation that 25% to 50% of them were lesioned by given pathological changes, while grade 3 represents the most severe changes and wide lesion-extension more than 50%. The evaluation of division into grades was made by three pathohistologists without detailed informations in advance about the present purpose.

Table 1 Comparison of renal Cd concentration and kidney weight between Cd-non-contaminated and Cd-contaminated groups of wild great bandicoots which are classified into small (S), medium (M) and large (L) subgroups based on body weights.

Group	Size (N)	Body weight (g)	Renal weight (g)	Cd concentration (μg/kg)
Non-Cd-contaminated	S (6)	174.17 ± 8.98	0.67 ± 0.05	70.81 ± 21.35
	M (5)	344.00 ± 19.39*	0.97 ± 0.06*	102.87 ± 38.19*
	L (10)	521.00 ± 13.45*	1.86 ± 0.17*	696.78 ± 148.96*
Cd-contaminated	S (37)	194.59 ± 5.19	0.88 ± 0.03#	73.54 ± 8.45
	M (16)	326.86 ± 6.17*	1.30 ± 0.05*, #	972.00 ± 250.92*, #
	L (17)	528.82 ± 11.34*	2.69 ± 0.17*, #	1800.77 ± 421.94*, #

Values represent mean ± SEM. Significantly different at $P < 0.05$, * compared to S size and # compared to non contaminated group

3.3.1. necrosis in renal cortex

In the Cd-non-contaminated area, about four fifths of small-sized animals belonged to grade 0, and about one fifth belonged to grade 1. All medium-sized animals belonged to grade 0. About one tenth of large-sized animals each belonged to grade 1 and grade 2 with the remaining eight tenth belonged to grade 0 (Table2).

In the Cd-contaminated area, about three quarters of small-sized animals belonged to grade 0 and more than one tenth belonged to grade 1, less than one tenth to grade 2 and the remaining to grade 3. As for medium-sized animals, most of them belonged to grade 0 and the remaining belonged to grade 1. On the other hand, as for large-sized animals, about one third of them belonged to grade 3, less than one tenth belonged to grade 2, more than one tenth belonged to grade 1, and only one half belonged to grade 0 (Table2).

3.3.2. infiltration of inflammatory cells

In the Cd-non-contaminated areas, about a half of the small-sized animals belonged to grade 0 and the rest half grade 1. Less than a half of the medium-sized animals belonged to grade 0 and a similar population of them belonged to grade 1 and the remainder belonged to grade 2. More than half of the large-sized animals belonged to grade 0, while one fifth each of them grade 1, and one tenth grade 2 and grade 3.

In Cd-contaminated areas, more than two thirds of the small-sized bandicoots belonged to grade 0 and about one fifth of them belonged to grade 1, a few of them to grade 2 and grade 3. As for medium-sized bandicoots, most of them belonged to grade 0 and a few belonged to grade 1. On the other hand, as for large-sized bandicoots, approximately one third each of them belonged to grade 0, grade 1 and grade 3, and a few of them belonged to grade 2.

Among bandicoots in Cd-non-contaminated areas, a half of the small-sized bandicoots each belonged to grade 0 and grade 1. More than a half of the medium-sized animals were assigned to grade 0, about one fifth each assigned to grade 1 and grade 2 without assignment to grade 3. More than two thirds of the large-sized animals were assigned to grade 0 and about one third to grade 2, without assignment to grade 1 or grade 3 (Table 2).

Among bandicoots in Cd-contaminated areas, about two thirds of the small-sized animals belonged to grade 0, about one third to grade 1, and a few to grade 3. More than a half of the medium-sized animals were assigned to grade 0, about one third to grade 1, and a few to grade 3 without assignment to grade 2. About one quarter of the large-sized animals were assigned to grade 0, about one third to grade 1, about one fifth to grade 2 and about a fourth to grade 3 (Table 2).

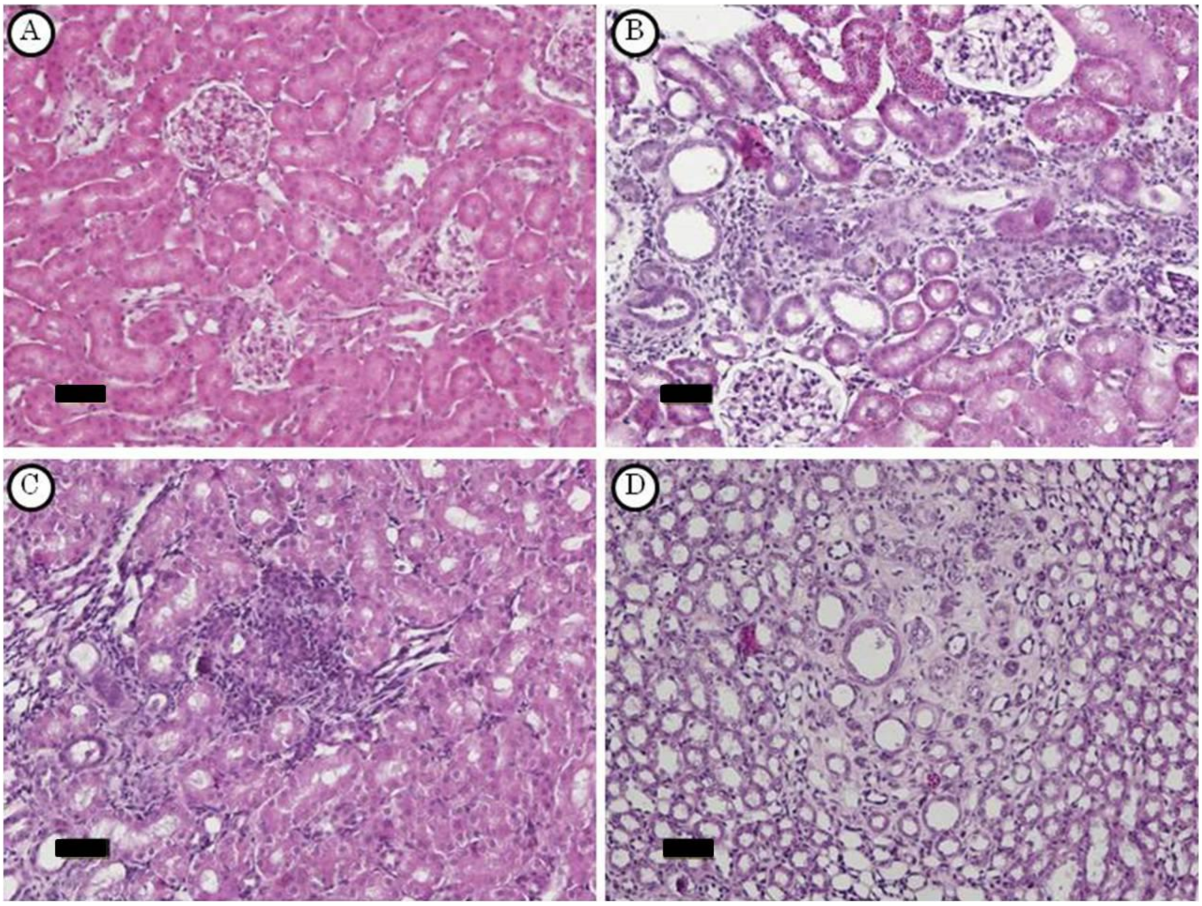

Figure 1 Light micrographs of renal pathological changes of great bandicoots. A) normal control, B) necrosis in cortex; grade 3, C) inflammatory cell infiltration; grade 3, D) fibrosis in medulla; grade 3. Bars represent 50 µm.

3.3.3. fibrosis in renal medulla

Table 2 Frequencies of occurrence of three histopathological changes and their grades among Cd-non-contaminated and Cd-contaminated groups of great bandicoots, each of which is classified into small, medium or large subgroups.

Histopathologies	Grade	Cd-non-contaminated group			Cd-contaminated group		
		S-size N=6	M-size N=5	L-size N=10	S-size N=37	M-size N=16	L-size N=17
		N/(%)	N/(%)	N/(%)	N/(%)	N/(%)	N/(%)
Necroses in	0	5/(83.33%)	5/(100%)	8/(80%)	8/(75.68%)	15/(93.75%)	9/(52.94%)
renal cortex	1	1/(16.67%)	0/(0%)	0/(0%)	5/(13.51%)	1/(6.25%)	2/(11.77%)
	2	0/(0%)	0/(0%)	1/(10%)	3/(8.11%)	0/(0%)	1/(5.88%)
	3	0/(0%)	0/(0%)	1/(10%)	1/(2.70%)	0/(0%)	5/(29.41%)
Inflammatory	0	3/(50%)	2/(40%)	6/(60%)	27/(72.97%)	15/(93.75%)	6/(35.29%)
cells infiltration	1	3/(50%)	2/(40%)	2/(20%)	7/(18.92%)	1/(6.25%)	5/(29.41%)
	2	0/(0%)	1/(20%)	1/(10%)	2/(5.41%)	0/(0%)	1/(5.89%)
	3	0/(0%)	0/(0%)	1/(10%)	1/(2.70%)	0/(0%)	5/(29.41%)
Fibrosis in	0	3/(50%)	3/(60%)	7/(70%)	22/(59.46%)	9/(56.25%)	4/(23.53%)
renal medulla	1	3/(50%)	1/(20%)	0/(0%)	13/(35.14%)	6/(37.5%)	6/(35.29%)
	2	0/(0%)	1/(20%)	3/(30%)	0/(0%)	0/(0%)	3/(17.65%)
	3	0/(0%)	0/(0%)	0/(0%)	2/(5.40%)	1/(6.25%)	4/(23.53%)

3.4. Expression of metallothionein (MT) genes, MT1A and MT2A, in kidney

The expression patterns of MT1A transcripts in any of the three body-sized subgroups were similar between the Cd-non-contaminated and Cd-contaminated groups with the highest expressions in the medium-sized subgroups and the least in the small-sized ones. In contrast, the expression pattern of MT2A in the Cd-contaminated group showed a tendency of animal size-depending decrease (Figure. 2), while the Cd-non-contaminated group has a pattern similar to that of the expression pattern for MT1A as described above (Figure. 2). However, no significant statistical differences in the gene expression levels were discerned for MT1A or MT2A between Cd-non-contaminated and Cd-contaminated groups in any of the three body-sized subgroups, and between any body-sized subgroups in either group (Figure. 2).

3.5. Discussion

The concentration of Cd (0.026 mg/kg) in rice grains from the Cd-non-contaminated area, Kalasin, Thailand, does not exceed the CCFAC (Codex Committee on Food Additives and Contaminants)-maximum permission level for Cd, 0.2 mg/kg, in rice grains [11]. In comparison, one (2.065 mg/kg) of the two Cd-concentrations in rice grains from the Cd-contaminated area was about 10 times higher than the maximum permission level for rice grains, whereas the other (0.146 mg/kg) is not over the maximum permission level, but exceeds the level (0.1 mg/kg) considered to be safe for staple food [13] & [20]. On the other hand, the Cd-concentration of soils from the Cd-non-contaminated area (range from non-detectable to 0.019 mg/kg) is very low as compared with the European Union (EU) Maximum Permissible (MP) concentration of soil-Cd （3.0 mg/kg) (10). and also does not exceed the Thai investigation level for soil-Cd (0.15 mg/kg) [12]. In contrast, the Cd concentrations in soil from Cd-contaminated area (0.217 mg/kg ~ 49.008 mg/kg) exceed the European Union Maximum Permissible, and extremely higher than the Thai investigation level for soil Cd.

In accord with previous studies [21] & [22] reporting that animals living in high Cd-contaminated areas exhibit high Cd-levels in the kidney of other animals, the present study revealed that the renal Cd-concentration was remarkably high in the large- and medium-sized subgroups of wild bandicoots from the Cd-contaminated areas. Among them the concentration of the large-sized subgroup was the highest among the total six subgroups, although the trend of higher concentration was found also in larger-sized subgroup from the Cd-non-contaminated area. It seems natural to consider that larger animals represent older ones in general. It is therefore expected that the effect of environmental Cd-exposure is much more evident in the large-sized animals than the small- and medium-sized ones in the Cd-contaminated areas.

In accord with this consideration, the present study revealed most severe pathological changes in the large-sized animals from the Cd-contaminated areas, although older rats are in general known to have higher incidence of renal fibrosis than younger counterparts [23]. This was also supported by the previous finding that Cd was accumulated in the body of other animals and humans for many years of living [24]. On the other hand, little differences in the occurrence of pathological changes were detected in the kidney between the small-sized subgroup of animals from the Cd-

Contaminated areas and the counterpart from the Cd-non-contaminated area. This feature suggest that younger animals are less susceptible to Cd-intoxication, or that they have a mechanism specific to the age for development of the intoxication effect, although it may simply represent shorter exposure of the specimens to Cd during their young life.

Considering that MT is a main intrinsic Cd-detoxifying molecule available to several organs including kidney [7] & [25]. and that the levels of MT molecule are usually correlated with Cd levels in experimental animals [26], the present authors had expected before experiment to observe some increased levels of transcription for MT1A and MT2A in the kidney of bandicoots, especially the large- and medium-sized subgroups, in the Cd-contaminated areas. However, the present study showed no significant relationship between the Cd-concentration and the MT-gene expression in the kidney. This is compatible with the previous finding that, in bank voles exposed to heavy metals in the wild environment, the level of MT2 gene expression was not positively correlated with Cd-levels in the kidney, though positively with the Cd-levels in the liver [22]. Nevertheless, there has been a study reporting positive effects of the Cd-level on MT1 gene expression in various organs including the kidney of bank voles environmentally exposed to Cd [22]. The species difference may be attributed to the discrepancy.

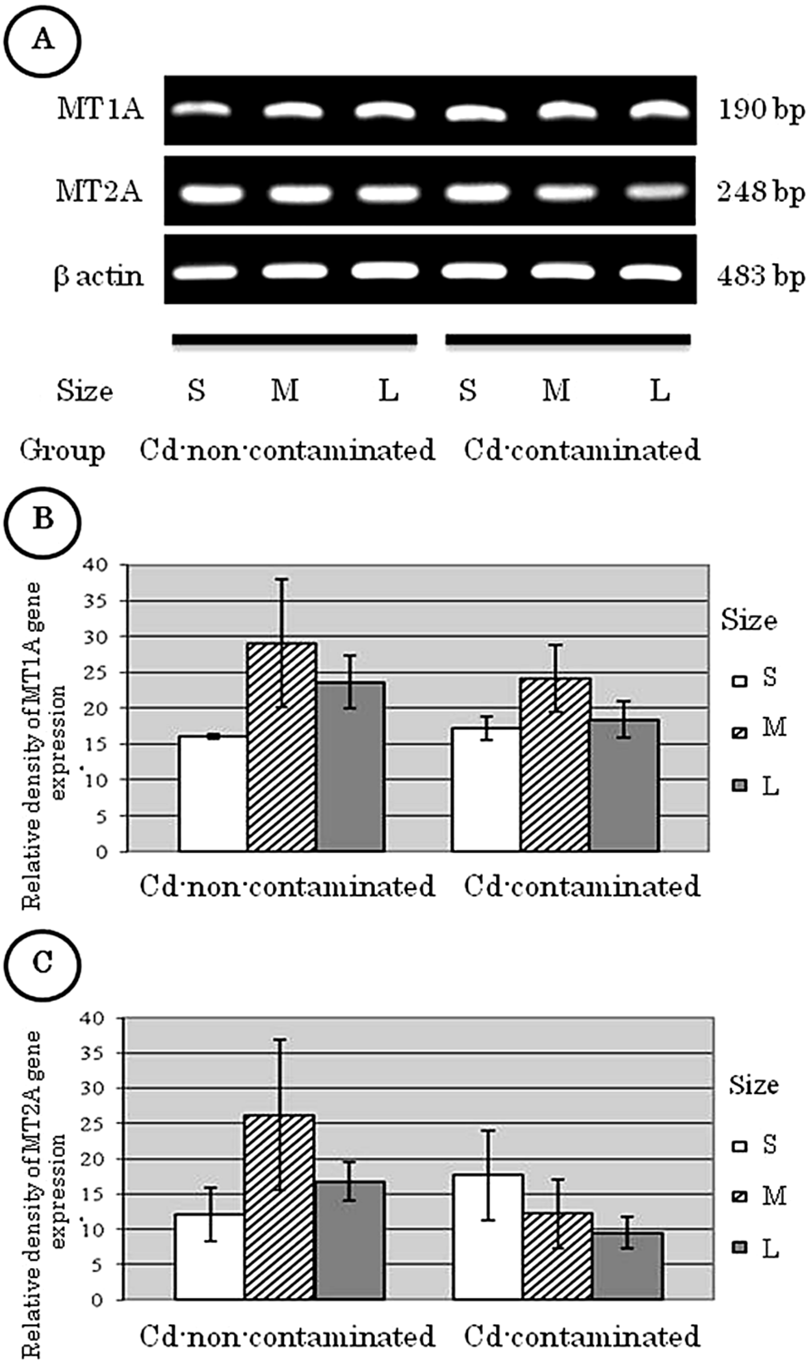

Figure 2 Comparison in RT-PCR of expression of MT1A and MT2A genes in the renal tissues of wild great bandicoots between Cd-non-contaminated and Cd-contaminated groups. A) RT-PCR products for MT1A and MT2A genes in electrophoresis and photograph with β-actin house keeping gene as the internal control. B, C) Individual columns represent relative intensities of MT1A and MT2A genes expressions which are corrected by corresponding β-actin bands with mean±SD. No significant differences between Cd-non-contaminated and Cd-contaminated groups ($p>0.05$).

No significant differences in the gene expression for MT in any body-sized subgroups between bandicoots from the Cd-contaminated areas and those from the Cd-non-contaminated area, together with no correlation of the MT levels with the Cd levels, suggest that Cd may employ other molecules to protect renal cells from the Cd-intoxication in the wild animals. One candidate molecule for the metal detoxification is glutathione. Swiergosz-Kowalewska *et al.*, [22] have indicated that the most sensitive parameter of metal toxicity for animals living in a chronically contaminated environment is the ratio of glutathione/glutathione disulfide and that the ratio is lowered in tissues and organs including the kidney of bank voles from other heavy metal-contaminated areas. There has also been a study showing that the elevation of glutathione level in the renal cortex reduces the nephrotoxicity in laboratory mice [22]. On the other hand, Misra *et al*., [27] found the expression of gene for MT increased in correlation with the renal Cd-levels in mice, but not rats, and that the basal expression level of gene for MT in the kidney of rats was high enough not to be further induced by Cd. Since the present wild great bandicoot is one strain of rats, it is necessary to examine the levels of glutathione in the kidney of the present animals.

4. Conclusion

The Cd concentration in the kidney in large-sized animals from the Cd-contaminated area was exceedingly high among all the specimens, while that in medium-sized animals from the Cd-contaminated area was higher than that in large-sized ones from the Cd-non-contaminated area. However, no significant difference was noted in the Cd-concentration between small-sized animals from the Cd-contaminated area and those from the Cd-non-contaminated area. In accord with the comparison result of the renal Cd-concentration, histopathological changes were more severe in the kidney of animals with larger body-sizes from the Cd-contaminated areas, although, no significant difference was noted in the expression of gene for metallothionien between kidneys of animals from Cd-contaminated and Cd-non-contaminated areas. These findings suggest that Cd-contamination could directly affect animals residing in those contaminated area with the age increasing, and that it may cause pathological changes of some organs directly/indirectly. These changes in the animals could be a bio-indicator for human who reside in the contaminated area.

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6. References

[1] Martelli, A., Rousselet, E., Dycke, C., Bouron, A., Moulis, J.M., 2006. Cadmium toxicity in animal cells by interference with essential metals. Biochimie 88, 1807-1814.

[2] Nordberg, G.F., Nogawa, K., Nordberg, M., Friberg, L.T., 2007. Chapter 23 - Cadmium. In: Gunnar FN, Bruce AF, Monica N, Lars T, FribergA2 -Gunnar F, Nordberg BAFMN, Lars TF, (Eds.) Handbook on the Toxicology of Metals. 3rd ed. Academic Press, Burlington.

[3] Godt, J., Scheidig, F., Grosse-Siestrup, C., Esche, V., Brandenburg, P., Reich, A., Groneberg, D.A., 2006. The toxicity of cadmium and resulting hazards for human health. Journal of Occupational Medicine and Toxicology 10, 1-22.

[4] Barbier, O., Jacquillet, G., Tauc, M., Cougnon, M., Poujeol, P., 2005. Effect of heavy metals on, and handling by, the kidney. Nephron Physiology 99, 105-110.

[5] Thevenod, F., 2003. Nephrotoxicity and the proximal tubule. Insights from cadmium. Nephron Physiology 93, 87-93.

[6] Klaassen, C.D., Liu, J., Choudhuri, S., 1999. Metallothionein: an intracellular protein to protect against cadmium toxicity. Annual Review of Pharmacology and Toxicology 39, 267-294.

[7] Garrett, S.H., Somji, S., Todd, J.H., Sens, M.A., Sens, D.A., 1998. Differential expression of human metallothionein isoform I mRNA in human proximal tubule cells exposed to metals. Environmental Health Perspectives 106, 825-831.

[8] Bylander, J.E., Li, S.L., Sens, M.A., Sens, D.A., 1995. Exposure of human proximal tubule cells to cytotoxic levels of $CdCl_2$ induces the additional expression of metallothionein 1A mRNA. Toxicology Letters 76, 209-217.

[9] Lu, J., Jin, T., Nordberg, G., Nordberg, M., 2005. Metallothionein gene expression in peripheral lymphocytes and renal dysfunction in a population environmentally exposed to cadmium. Toxicology and Applied Pharmacology 206, 150-156.

[10] Swaddiwudhipong, W., Limpatanachote, P., Mahasakpan, P., Krintratun, S., Padungtod, C., 2007. Cadmium-exposed population in Mae Sot District, Tak Province: 1. Prevalence of high urinary cadmium levels in the adults. Journal of the Medical Association of Thailand 90, 143-148.

[11] Simmons, R.W., Pongsakul, P., Saiyasitpanich, D., Klinphoklap, S., 2005. Elevated levels of cadmium and zinc in paddy soils and elevated levels of cadmium in rice grain downstream of a zinc mineralized area in Thailand: implications for public health. Environmental Geochemistry and Health 27, 501-511.

[12] Zarcinas, B., Pongsakul, P., McLaughlin, M., Cozens, G., 2004. Heavy metals in soils and crops in Southeast Asia 2. Thailand. Environmental Geochemistry and Health 26, 359-371.

[13] Satarug, S., Haswell-Elkins, M.R., Moore, M.R., 2000. Safe levels of cadmium intake to prevent renal toxicity in human subjects. British Journal of Nutrition 84, 791-802.

[14] Boonsong, L., McNeely, J.A., Marshall, J.T., 1988. Mammals of Thailand. Association for the Conservation of Wildlife. Bangkok, Thailand, Kuruspha Ladprao Press.

[15] Sakagami, H., Suzuki, H., Kamata, A., Owada, Y., Fukunaga, K., Mayanagi, H., Kondo, H., 2006. Distinct spatiotemporal expression of EFA6D, a guanine nucleotide exchange factor for ARF6, among the EFA6 family in mouse brain. Brain Research1093, 1-11.

[16] Hamada, T., Nakano, S., Iwai, S., Tanimoto, A., Ariyoshi, K., Koide, O., 1991. Pathological study on beagles after long-term oral administration of Cadmium. Toxicologic Pathology 19, 138-147.

[17] Jihen el, H., Imed, M., Fatima, H., Abdelhamid, K., 2008. Protective effects of selenium (Se) and zinc (Zn) on cadmium (Cd) toxicity in the liver and kidney of the rat: histology and Cd accumulation. Food and Chemical Toxicology 46, 3522-3527.

[18] Robbins, S.L., Kumar, V., Cotran, R.S., 2010. Robbins and Cotran pathologic basis of disease. Philadelphia, PA, Saunders/Elsevier.

[19] Salinska, A., Wlostowski, T., Zambrzycka, E., 2012. Effect of dietary cadmium and/or lead on histopathological changes in the kidneys and liver of bank voles Myodes glareolus kept in different group densities. Ecotoxicology 21, 2235-2243.

[20] Teeyakasem, W., Nishijo, M., Honda, R., Satarug, S., Swaddiwudhipong, W., Ruangyuttikarn, W., 2007. Monitoring of cadmium toxicity in a Thai population with high-level environmental exposure. Toxicology Letters 169, 185-95.

[21] Satarug, S., Baker, J.R., Reilly, P.E., Moore, M.R., Williams, D.J., 2002. Cadmium levels in the lung, liver, kidney cortex, and urine samples from Australians without occupational exposure to metals. Archives of Environmental Health 57, 69-77.

[22] Swiergosz-Kowalewska, R., Bednarska, A., Callaghan, A., 2007. Expression of metallothionein genes I and II in bank vole Clethrionomys glareolus populations chronically exposed in situ to heavy metals. Environmental Science & Technology 41, 1032-1037.

[23] Percy, C.J., Brown, L., Power, D.A., Johnson, D.W., Gobe, G.C., 2009. Obesity and hypertension have differing oxidant handling molecular pathways in age-related chronic kidney disease. Mechanisms of Ageing and Development 130, 129-38.

[24] Akesson, A., Lundh, T., Vahter, M., Bjellerup, P., Lidfeldt, J., Nerbrand, C., Samsioe, G., Strömberg, U., Skerfvin, S., 2005. Tubular and glomerular kidney effects in Swedish women with low environmental cadmium exposure. Environmental Health Perspectives 113, 1627-1631.

[25] Hermesz, E., Abraham, M., Nemcsok, J., 2001. Tissue-specific expression of two metallothionein genes in common carp during cadmium exposure and temperature shock. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology 128, 457-465.

[26] Geffard, A., Geffard, O., His, E., Amiard, J.-C., 2002. Relationships between metal bioaccumulation and metallothionein levels in larvae of Mytilus galloprovincialis exposed to contaminated estuarine sediment elutriate. Marine Ecology Progress Series 233, 131-142.

[27] Misra, R.R., Crance, K.A., Bare, R.M., Waalkes, M.P., 1997. Lack of correlation between the inducibility of metallothionein mRNA and metallothionein protein in cadmium-exposed rodents. Toxicology 117, 99-109.